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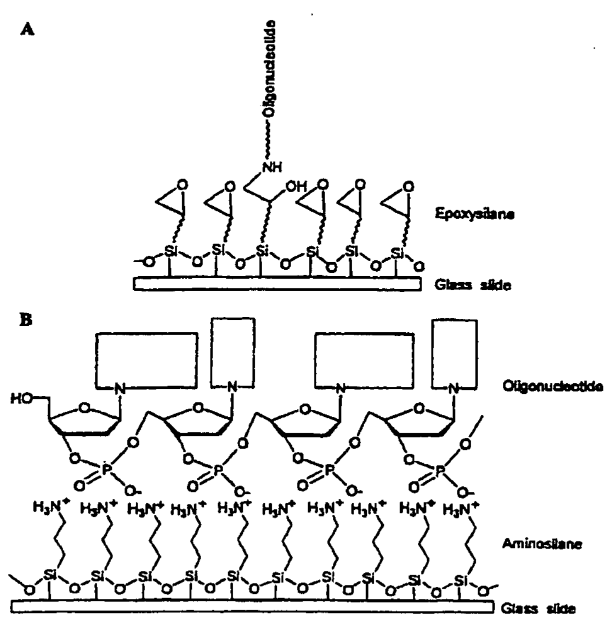

(57) Abstract: The invention provides novel methods for making and using array-based nucleic acid hybridization substrates comprising non-covalently associated nucleic acids. The invention also provides nucleic acid arrays comprising a solid surface comprising a plurality of discrete biosites comprising a non-covalently associated nucleic acid. The invention also provides a method for determining if a nucleic acid in a test sample can hybridize to a nucleic acid immobilized onto an array.

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ARRAYS COMPRISING NON-COVALENTLY ASSOCIATED NUCLEIC ACID PROBES AND METHODS FOR MAKING AND USING THEM

RELATED APPLICATIONS

This application incorporates by reference and claims the benefit of priority under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 60/143,926, filed July 14, 1999. The aforementioned application is explicitly incorporated herein by reference in its entirety and for all purposes.

TECHNICAL FIELD

This invention relates to molecular biology, genetic diagnostics and nucleic acid array, or "biochip," technology. In particular, the invention provides novel methods and compositions for making and using array-based nucleic acid hybridization substrates.

BACKGROUND

The birth of DNA microarray technology and its ability to collect massive amounts of data in a short period of time is one of the most exiting events in DNA science in recent years. Based upon the use of such solid phase arrays of nucleic acid probes, large scale mutational analysis and the analysis of gene expression at the mRNA level is becoming a reality. The key to these advances has been the development of immobilization chemistry for the spatially resolved attachment of DNA probes to the solid support, so as to form the desired microarrays.

In spite of the fact that adsorptive attachment of probes to fibrous nitrocellulose or nylon membranes has been used for many years as a standard hybridization technique, research in the microarray field has been concentrated on the development of covalent coupling of DNA probes to the planar surfaces. Such covalent coupling requires activation of the underlying planar surface with cross-linking reagents and/or modification of the DNA molecule with a reactive group.

It was suggested that if a DNA probe molecule were adsorbed to the surface by multiple constraining contacts, it would serve as a poor hybridization probe, due to the loss of configurational freedom and the attendant loss of the capacity to form a double helix between target and probe. If few adsorptive contacts were made between probe and the surface, the non-covalently attached DNA molecule would be susceptible to removal from

the surface. Therefore, the state of the art in the DNA microarray field has been based upon covalent attachment strategies.

It is known that unmodified oligonucleotides, 10 to 40 bases in length, can adsorb in a slowly reversible fashion to surfaces that have been coated with cationic groups tethered at the end of a short molecular chain, such as a chain 2 to 10 bonds in length. It is also known that, once adsorbed to the cationic surface in that way, oligonucleotides retain the capacity to bind a cognate nucleic acid strand. (see, e.g., L.A. Chrisey, et al. U.S. Patent No. 5,688,642; Balladur (1997) J. Colloid Interface Science 194:408-418). However, only low specificity hybridization could be shown by previous implementations of such an adsorptive approach.

SUMMARY

The invention provides a method of producing an array comprising a plurality of discrete biosites, wherein the biosites comprise non-covalently attached nucleic acids, comprising the following steps: (a) providing a solid surface comprising a net positive charge or derivatized with a composition comprising a net positive charge; (b) providing at least one solution comprising a nucleic acid comprising a net negative charge; (c) depositing a solution of step (b) onto a discrete biosite on the solid support of step (a), wherein the nucleic acid becomes non-covalently attached to the solid support of step (a) at least in part by an electrostatic attraction between the negatively charged nucleic acid and the positively charged solid support; and, (d) contacting the solid support of step (c) with a composition that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid. In one embodiment, the method further comprises drying the solid support after step (c) and before step (d).

In alternative embodiments of the invention, the solid surface can comprise a glass surface. The glass surface can comprise a planar glass surface, a glass bead, a porous glass surface or a surface comprising a plurality of glass fibers or equivalents. The solid surface can also comprise a nitrocellulose or a nylon membrane or equivalents.

In one embodiment of the methods of the invention, substantially all of the solid surface of step (a) is positively charged. Alternatively, the solid surface of step (a) can be positively charged substantially only on a plurality or a subset of discrete biosites.

In one embodiment of the methods of the invention, the solid surface of step (a) can comprise an amino-derivatized solid surface. The amino-derivatized solid surface can comprise an aminosilane-derivatized solid surface. In alternative embodiments, the aminosilane-derivatized solid surface can comprise an alkine-bridged bis-(aminosilane) or tris-(aminosilane) or an equivalent thereof or a mixture thereof. The aminosilane can comprise a 3-aminopropyltriethoxysilane, a N-(2-aminoethyl)-3-aminopropyltrimethoxysilane or an equivalent thereof or a mixture thereof.

In one embodiment of the methods of the invention, the solid surface of step (a) comprises a cationic polyacrylamide surface. The cationic polyacrylamide surface can comprise an acrylamide/aminoethylmethacrylate composition, a methyl-aminoethylmethacrylate, a trimethylaminoethylmethacrylate, an amino-hexylmethacrylate, an aminopropyl acrylamide, a methyl aminopropyl acrylamide, a dimethyl aminopropyl acrylamide or a trimethyl aminopropyl acrylamide, or mixtures or equivalents thereof.

In one embodiment of the methods of the invention, the solid surface of step (a) comprises a cationic hydrogel. The cationic hydrogel can comprise a poly(vinyl alcohol) - poly (ally-biguanido-co-allylamine) hydrochloride composition.

In another embodiment, the solid surface of step (a) comprises a diaminocyclohexane (DACH) plasma polymer, or, the solid surface of step (a) comprises a polystyrene treated by oxidative amination.

The solid surface of step (a) can also comprise a solid surface derivatized with a positively charged protein. The positively charged protein can comprise a protamine, a polyamine, a homopolyamino polypeptide or a co-polyamino polypeptide or an equivalent thereof. The protein can also comprise a spermine, a polyornithine, a polylysine, a polyarginine, or a co-poly-(Lys, Ala) acid or an equivalent thereof.

The solid surface of step (a) can also comprise a solid surface derivatized with a cationic lipid or detergent or an equivalent thereof. The cationic lipid or detergent can comprise a trimethylammonium group or an equivalent thereof.

In one embodiment, the solution of step (b) comprises an aqueous solution. The aqueous solution can consist essentially of distilled water.

In one embodiment, the nucleic acid comprises a cDNA or a genomic DNA. The cDNA or genomic DNA can comprise a length of between about 100 bases to about 800

bases. Alternatively, the nucleic acid can comprise an oligonucleotide. The oligonucleotide can comprise a length of between about 8 bases to about 80 bases or a length of between about 10 bases to about 15 bases. The concentration of nucleic acid in the aqueous solution can be between about 0.2 μM to about 25 μM, between about 5 μM to about 20 μM, or between about 9 μM to about 10 μM.

In one embodiment of the methods, the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid neutralizes the positive charges by a covalent reaction. This positive charge-neutralizing composition can form a Schiff base with a positively charged composition on the surface of the array. Alternatively, the positive charge-neutralizing composition can comprise an aldehyde or a ketone.

In one embodiment, the positive charge-neutralizing composition can comprise an acylating reagent. In alternative embodiments, the reaction of the acylating reagent with a positively charged composition on the solid support can produce a carboxamide, a sulfonamide, a urea or a thiourea. The acylating reagent can comprise an acetic anhydride or a butyric anhydride. The acetic anhydride or the butyric anhydride can be in a vapor phase. The reaction conditions of step (d) can comprise a temperature of about 50°C and an atmospheric pressure of about 22 mM of Hg. The reaction conditions can last for about 8 to 18 hours.

One embodiment of the methods of the invention further comprises the step of reacting the solid support of step (d) with a succinic anhydride or equivalent thereof. The reaction conditions can comprise 0.5 M succinic anhydride in DMF at room temperature for about one hour.

In one embodiment, the acylating reagent comprises a methylisothiocyanate or equivalent thereof.

In the methods of the invention the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid of step (d) can neutralize positive charge by electrostatic neutralization. In one embodiment, the composition comprises a high molecular weight polymer comprising a net negative charge. The high molecular weight polymer can comprise a polysaccharide, a polysulfate, a dry milk or a casein or a nucleic acid with a net negative

charge, or equivalent thereof. The nucleic acid with a net negative charge can be salmon sperm DNA.

In the methods of the invention the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid of step (d) can comprise a basic pH buffer. The buffer can have a pH of between about 9 and 9.5.

In the methods of the invention the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid of step (d) can comprise a detergent comprising a net negative charge.

In the methods of the invention the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid of step (d) can neutralize the positive charge by non-covalent and non-electrostatic adsorption to the solid surface, thereby masking substantially all of the exposed positive charges on the array surface. In this embodiment, the composition can comprise a high molecular weight polymer with no net positive or negative charge. In this embodiment, the high molecular weight polymer can be Denhardt's solution, a neutral detergent solution a serum albumin or a polyvinylpyrrolidone, or an equivalent thereof. The polyvinylpyrrolidone can comprise a N-vinyl pyrrolidone, a 3-methyl N-vinylpyrrolidone, a N-vinyl amide pyrrolidone, a N-vinyl acetate pyrrolidone, a vinylpyrrolidone -vinyl acetate copolymer, or an acrylamide- vinylpyrrolidone co-polymer, or an equivalent thereof or a mixture thereof.

The invention also provides a nucleic acid array comprising a solid surface comprising a plurality of discrete biosites comprising a non-covalently associated nucleic acid produced by a method comprising the following steps: (a) providing a solid surface comprising a net positive charge or derivatized with a composition comprising a net positive charge; (b) providing at least one solution comprising a nucleic acid comprising a net negative charge; (c) depositing a solution of step (b) onto a discrete biosite on the solid support of step (a), wherein the nucleic acid becomes non-covalently attached to the solid support of step (a) at least in part by an electrostatic attraction between the negatively charged nucleic acid and the positively charged solid support; and, (d) contacting the solid

support of step (c) with a composition that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid. The array surface onto which the nucleic acid has been deposited can be coated with a monolayer of adsorbed, non-covalently attached nucleic acid. The monolayer of adsorbed, non-covalently attached oligonucleotides can have an oligonucleotide density of about 10^7 to about 10^{12} molecules per mm^2, or, an oligonucleotide density of about 10^{11} molecules per mm^2. The nucleic acid can comprises a cDNA or a genomic DNA. The cDNA or genomic DNA can comprise a length of between about 50 bases to about 1000 bases, between about 100 bases to about 800 bases, or between about 200 bases to about 600 bases. The nucleic acid can comprise an oligonucleotide. The oligonucleotide can comprise a length of between about 8 bases to about 80 bases or a length of between about 10 bases to about 15 bases. The DNA array solid surface can comprise a glass surface. The glass surface can comprise a planar glass surface, a porous glass surface or a surface comprising a plurality of glass fibers or equivalent thereof. The solid surface can comprise a nitrocellulose or a nylon membrane or equivalent thereof.

In one embodiment of the DNA array, the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid neutralizes the positive charges by a covalent reaction. The positive charge-neutralizing composition can comprise an acylating reagent or equivalent thereof. The acylating reagent can comprise an aldehyde or a ketone. The acylating reagent can comprise an acetic anhydride or a butyric anhydride or equivalent thereof. The reaction of the acylating reagent with a positively charged composition on the solid support can produce a carboxamide, a sulfonamide, a urea or a thiourea or equivalent thereof.

The invention also provides a method for determining if a nucleic acid in a test sample can hybridize to a nucleic acid immobilized onto an array comprising the following steps: (a) providing a test sample comprising a nucleic acid; (b) providing a nucleic acid array comprising a solid surface comprising a plurality of discrete biosites comprising a non-covalently associated nucleic acid produced by the following steps:

(i) providing a solid surface comprising a net positive charge or derivatized with a composition comprising a net positive charge;

(ii) providing at least one solution comprising a nucleic acid comprising a net negative charge;

(iii) depositing a solution of step (ii) onto a discrete biosite on the solid support of step (a), wherein the nucleic acid becomes non-covalently attached to the solid support of step (a) at least in part by an electrostatic attraction between the negatively charged nucleic acid and the positively charged solid support, and,

(iv) contacting the solid support of step (iii) with a composition that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid; and,

(c) contacting the test sample of step (a) with the DNA array of step (b); and (d) determining if a nucleic acid in the test sample hybridizes to a nucleic acid immobilized onto an array.

In the current invention demonstrates that subsequent to adsorptive binding to a solid surface comprising a net positive charge or derivatized with a composition comprising a net positive charge (e.g. a short-chain cationic surface), nucleic acids (e.g., cDNAs) or oligonucleotides can be made to form a dense monolayer upon the surface. The nucleic acid or oligonucleotide density on these surfaces approaches the theoretical limit, i.e., about 40% of the available surface area. The invention also demonstrates that the resulting nucleic acid- or oligonucleotide-coated surface can be secondarily modified, or "capped", with any of a number of covalent or non covalent charge-modifying (e.g., amine-modifying), charge-neutralizing or charge-masking reagents before use of the array for hybridization to a nucleic acid is a test sample.

In alternative embodiments, the hybridization can be under high salt and/or high pH buffered conditions to reduce, or even eliminate, the overall excess positive charge density on the surface, without displacing the non-covalently bound nucleic acid or oligonucleotide of interest (the nucleic acid or oligonucleotide "probe" non-covalently adsorbed to a biosite).

The invention demonstrates that "capping," neutralizing or masking "residual" positive charges on the nucleic acid- or oligonucleotide-coated monolayer surface (i.e., positive charges not involved in non-covalent association with the nucleic acid probe) "develops" the capacity for high resolution and high affinity nucleic acid hybridization to

both low and high molecular weight nucleic acid targets. In the absence of "capping," neutralizing or masking the "residual" positive charges (e.g., by a chemical capping step), the oligonucleotide-modified cationic surface binds nucleic acid targets in a non-specific fashion. This may be due to an overabundance of excess cationic charge. In contrast, the methods of the invention make and use an array comprising non-covalently associated nucleic acids probes on a surface in which sufficient "residue" positive charges are "capped," neutralized or masked to convert an other-wise unusable, nonspecific surface to a surface capable of specific hybridization with a test sample.

The methods of the invention also provide an easy and reproducible means to make an array comprising a dense monolayer of non-covalently adsorbed nucleic acids or oligonucleotides on a positively charged solid surface (e.g., a short chain cationic surface). The array produced by the methods of the invention forms a monolayer that is a structure capable of extremely high selectivity in nucleic acid hybridization reactions. This high affinity nucleic acid hybridization is accomplished by secondary chemical or "electrostatic" modification or "masking" of the "residual" surface positive charges (e.g., charged amines) not directly involved in association with the adsorbed nucleic acid or oligonucleotide probe.

Before this invention, it was thought that high selectivity binding of target to such adsorbed probes could not be achieved. The novel chemical or adsorptive or masking "capping" step had not been previously employed. In fact, before this invention, several experts stated that adsorptive binding to short chain coatings would not be selective enough to be useful for hybridization in a microarray format, see, e.g., Duggan (1999) Nature Genetics Supplement 21:10-14.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

All publications, patents and patent applications cited herein are hereby expressly incorporated by reference for all purposes.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1A is a schematic illustrating the chemistry of covalent binding of oligonucleotides to a glass surface. Figure 1B is a schematic illustrating the chemistry of non-covalent (adsorptive) oligonucleotide attachment to an amino-derivatized solid surface.

Figure 2A summarizes the results of a titration of an array of the invention, in particular, an array having a radiolabeled 12-mer oligonucleotide adsorbed to an aminosilanized surface, using a graph wherein the number of probes/mm^2 x 10^{10} is a function of concentration of printed probes in moles (M). Figure 2B summarizes the results of a binding experiment where a synthetic radiolabeled probe is hybridized to probe that is adsorbed to an aminosilanized surface.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

The invention provides an extremely simple and reproducible method for the production of DNA arrays by employing adsorptive, non-covalent attachment of nucleic acids and oligonucleotide probes to positively charged solid surfaces, e.g., aminosilanized glass surfaces. The method comprises two steps. First, nucleic acids (desired to act as immobilized probes) in a solution (e.g., a water solution) are deposited onto a positively charged solid surface, e.g., an aminosilanized glass substrate. In an array format, these deposits are typically in the form of a plurality of discrete spots, or "biosites." This is optionally followed by a drying step. Second, after electrostatic association of the probes to the array, the remaining positive charges not associated with a probe nucleic acid, i.e., the "residual" positive charges, are "capped" by reaction with a second composition, e.g., by acylation, or electrostatically neutralized with a composition comprising a negative charge, or, "masked" with an adsorbed composition having no net positive or negative charge (a "neutral" composition), e.g., a neutral lipid detergent or a neutral high MW polymer. For example, in one embodiment, "unused" or "residual" positively charged amines; i.e., those amines not involved in direct association with adsorbed probe molecules, are reacted with a compound that neutralizes the positive charge (e.g., an acylation reaction). After "capping" or neutralization or "masking" of the residual positive charges, the attached nucleic acid or oligonucleotide probes are not removed from the array surface under standard hybridization and washing conditions, including high salt and high pH treatments.

In spite of the fact that adsorbed nucleic acid or oligonucleotide is bound via multiple contacts to the surface and therefore may have lost configurational freedom required to form a perfect double helix with its cognate target, the product of such adsorptive coupling, followed by judicious capping, displays a specificity which is as high as that seen in a standard solution-state hybridization reactions, or, for surface hybridization to probes linked covalently to the surface at a single point.

To demonstrate the efficiency of probes non-covalently associated to arrays using the methods of the invention, the adsorptive strategy of the invention was compared to standard covalent strategies for immobilizing nucleic acid probes to arrays. Probes were attached to a planar glass substrate were using both methodologies and immobilized probe efficiency in hybridization reactions were compared. Specifically, as a covalent attachment model, amino-modified oligonucleotides were covalently reacted to an epoxysilanized surface (Figure 1). This is a well known method of choice for covalent attachment in microarray fabrication. The embodiment of the invention used incorporated the non-covalent attachment based on adsorption of an unmodified oligonucleotide to an amino-derivatized surface (Figure 2). See Example 1 for a detailed description of these studies.

For a positively charged surface covered with a monolayer of adsorbed probe, there are two independent processes that would cause target nucleic acid to bind to the surface: simple electrostatic attraction to the cationic surface and the desired process of duplex formation with to the attached probes. If there were a high density of amines on the surface, not involved in direct adsorptive interaction, electrostatic attraction between target and the surface could become a dominating force, thereby overwhelming the contribution of duplex formation and poor base sequence selectivity would be seen. Thus, the invention sets forth novel ways to "cap" the excess of unused surface cation, while retaining tight adsorptive interaction between probe and the surface.

In developing the invention, different classes of capping reagents were tried. In initial experiments, when short oligonucleotide targets were hybridized, non-covalent capping via prehybridization with SSC buffer at pH 7.5 with 5x Denhardt's solution, or SSC with 1 mg/ml of salmon sperm DNA was sufficient. However, when long PCR amplicons were used as targets, residual interaction with surface charge, not accounted for by that

simple adsorptive capping gave rise to poor specificity. Adsorptive capping with 1% poly(styrene sulfonic acid) made the surface too negative and unable to bind target.

Therefore, covalent capping reactions were investigated as an alternative. Several acylating reagents were tested, including acetic anhydride, butyric anhydride (both at 0.5 M in DMF) and methylisothiocyanate (0.5 M in 10% pyridine/DMF) for capping of amino silanized surfaces after probe coupling. Capping reactions were carried out at room temperature for 1 h. Due to incomplete covalent capping, the above reactions yielded a slightly positive surface which produced non-specific target binding at less than pH 7.5 after reaction with acyl anhydrides and at less than pH 8 after reaction with isothiocyanate.

Thus, as a secondary refinement of the above covalently capped surfaces, hybridization studies were performed at a higher pH: pH 8.5 and at 150 mM concentration of sodium. Under those conditions, good specificity of PCR amplicon hybridization was obtained for probes printed at about 6 μM. However, at higher printed probe concentrations, decreased specificity of hybridization was observed. A possible explanation for this observation being that the excess of printed oligonucleotides serves as an inhibitor of the capping reaction, leaving more uncapped amine during hybridization. In that instance, electrostatic attraction with target nucleic acid dominates during hybridization, causing target to bind to the amino-surface via a non nucleic acid mechanism.

Having to print oligonucleotides at such a narrow concentration optima is not desirable. Therefore, a neutral, slightly negatively and highly negatively charged surface was made to determine if it would broaden the above-mentioned range of optimal probe concentration. A neutral surface was obtained by capping with acetic anhydride in vapor phase at 50° C and 22 mM of Hg in a vacuum oven. Capping reactions were carried out 1 h and overnight. It was found that the overnight reactions significantly reduced electrostatic attraction and improved specificity. However, the 1 h reaction product retained a slightly positively charge and gave poor specificity for amplicon targets.

To make a slightly negatively charged surface, arrays that had been already capped with acetic anhydride were further processed by capping again with 0.5 M succinic anhydride in DMF at room temperature for 1 h. It was observed that such "double capped" arrays gave very good amplicon target binding specificity over a wide range of applied probe

concentrations (5 to at least 20 μM). In other words, specificity did not depend on the precise concentration of the probes printed.

A highly negatively charged surface was prepared by a one step reaction with succinic anhydride, as described above. However, this surface was found to be too negatively charged to support hybridization.

Based upon the above experience with covalent capping, it can be concluded that the general principle underlying the current invention is that capping the residual positive charges (or neutralizing or masking them) can produce a surface with a nearly neutral net surface charge. Thus, the invention provides an adsorptive capping (or neutralizing or masking) reaction. Modifications of solution state pH can be screened to reduce the net charge on the amino-silane groups of the surface.

The effect of high salt and high pH buffers on "unused" surface amines was also tested. It was found that a pre-wash step with 300 mM sodium carbonate buffer at pH 9.5 and 5x Denhardt's solution (adsorptive capping), followed by hybridization in same buffer produces a surface capable of extremely high selectivity and high affinity nucleic acid hybridization. Thus, optimal surface capping can be achieved via this extremely simple adsorptive capping method of the current invention.

A mutational analysis was performed by hybridization using arrays based on the above-described adsorptive oligonucleotide attachment methods. These results were compared with arrays prepared using traditional covalent binding chemistry. Both types of arrays were made with a Hamilton robot (see above). Results obtained using PCR amplicon targets to the two different types of arrays shows that specificity achieved using adsorptive probe binding, followed by optimal covalent or adsorptive capping is at least as good as the specificity of hybridization obtained with covalently attached probes.

There are several practical advantages to the current invention. First, the affinity and selectivity of non-covalently immobilized probe to sample target duplex formation is at least as good as the best that can be obtained by traditional methods of probe attachment. Second, the adsorptive methods of the invention can accommodate use of unlabeled probes at an applied concentration which is at least 5 times lower than required for other chemistries. As a result, the probe cost of manufacture is reduced at least ten fold. Lastly, because probe can be printed in distilled water (which denatures nucleic acid

secondary structure) and because probe binding is adsorptive, which tends to maximize surface contacts, probes affixed to a surface in this way tend to show smaller artifacts due to intramolecular folding interactions.

The mechanism by which such adsorbed probes bind to target nucleic acid is understood in general detail. The fact that target binding to such probes displays a high level of sequence selectivity and high overall binding affinity confirms that a double helix is formed upon binding. Probes bound to the surface in this way must be constrained by multiple contacts with the underlying surface; indeed, the prior art incorrectly suggested that such adsorbed probes would be unavailable to bind a cognate target.

Preliminary data and theoretical modeling suggest that duplex can still form with probes adsorbed by the methods of the invention because the probes are not bound rigidly to the underlying surface. Instead, they are "trapped" upon the planar surface, unable to diffuse off it. However, the adsorbed probes remain relatively free to engage in rotational and the other re-orientational motions with cognate target molecule to form a double helix. It is the existence of those two-dimensional degrees of freedom (on an appropriately capped surface) which is a physical basis for the current invention; however, the invention is not limited by any particular mechanism of action.

There are several practical advantages to the methods of the invention for microarray formation by adsorptive probe attachment. First, the affinity and selectivity of duplex formation are at least as good as the best that can be obtained by traditional methods of probe attachment. Second, the adsorptive method can use inexpensive, unmodified probes at an applied concentration that is at least about five times lower than that required for the currently available covalent attachment chemistries. As a result, the probe cost of manufacture can be reduced at least ten fold using the methods of the invention. Lastly, because probe can be "printed" (deposited on the positive surface of the array) in distilled water (which denatures nucleic acid secondary structure) and because binding is adsorptive, which tends to maximize surface contacts, probes affixed to a surface in this way may tend to show smaller artifacts due to intra-molecular folding interactions.

The invention demonstrates that subsequent to an appropriate capping reaction, target binding to probes non-covalently adsorbed by the methods of the invention display a high level of sequence selectivity to target. A high overall binding affinity was also

demonstrate. These results confirm that a double helix is being formed between immobilized probe and target nucleic acid. These data also suggest that when the probes non-covalently associate to the weakly cationic aminosilane surface they are not bound rigidly to the underlying surface. Instead, the probes are "trapped" upon the planar surface in a potential well, unable to diffuse off it, but relatively free to engage in rotation and the other re-orientational motions upon the surface. The movements facilitate formation of a double helix with a cognate DNA or RNA target.

DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

The term "aminosilane-derivatized solid surface" as used herein includes any solid surface covalently or non-covalently conjugated with an aminosilane moiety or equivalent, as described in detail below.

The terms "array" or "microarray" or "DNA array" or "nucleic acid array" or "biochip" as used herein means a plurality of target elements, each target element comprising a defined amount of one or more nucleic acid or polypeptide molecules, or probes (defined below), immobilized (including non-covalent associations, as described herein) to a solid surface; e.g., if nucleic acid is immobilized, the array can be used for hybridization to sample nucleic acids (e.g., oligonucleotides, RNA, cDNA, and the like) or polypeptides. The immobilized nucleic acids can contain sequences from specific messages (e.g., as cDNA libraries) or genes (e.g., genomic libraries), including substantially all of a subsection of (e.g., one or more chromosomes) or substantially all of a genome, including a human genome. Other target elements can contain reference sequences and the like. The term "probe(s)" or "nucleic acid probe(s)" as used herein, is defined to be a collection of one or more nucleic acid fragments (e.g., immobilized nucleic acid, e.g., a nucleic acid array) whose hybridization to a sample of target nucleic acid (defined below) can be detected. The target elements of the arrays may be arranged on the solid surface at different sizes and different densities. The target element densities will depend upon a number of factors, such as the nature of the label, the solid support, and the like. Each target element may comprise

substantially the same nucleic acid sequences, or, a mixture of nucleic acids of different lengths and/or sequences. Thus, for example, a target element may contain more than one copy of a cloned piece of DNA, and each copy may be broken into fragments of different lengths, as described herein. The length and complexity of the nucleic acid fixed onto the target element is not critical to the invention. The methods of this invention provide arrays comprising nucleic acids non-covalently immobilized on any solid or semi-solid surface (e.g., nitrocellulose, glass, quartz, fused silica, plastic, hydrogels, and the like). See, e.g., U.S. Patent No. 6,063,338 describing multi-well platforms comprising cycloolefin polymers if fluorescence is to be measured. In some embodiments, the methods of the invention can be practiced on variations of arrays of nucleic acids (in accordance to the embodiments of the invention described herein) as described, for instance, in U.S. Patent Nos. 6,087,112; 6,087,103; 6,087,102 ; 6,083,697; 6,080,858; 6,054,270; 6,045,996; 6,022,963; 6,013,440; 5,959,098; 5,856,174; 5,770,456; 5,700,637; 5,556,752; 5,143,854; see also, e.g., WO 99/51773; WO 99/09217; WO 97/46313; WO 96/17958; WO 89/10977; see also, e.g., Johnston (1998) Curr. Biol. 8:R171-R174; Schummer (1997) Biotechniques 23:1087-1092; Kern (1997) Biotechniques 23:120-124; Solinas-Toldo (1997) Genes, Chromosomes & Cancer 20:399-407; Bowtell (1999) Nature Genetics Supp. 21:25-32; Epstein (2000) Current Opinion in Biotech. 11:36-41.

The term “biosite” as used herein means a discrete area, spot or site on the top (a solid or “semi-solid” surface) of the array, or base material, comprising a non-covalently immobilized probe. Typically, arrays comprise a plurality of such “biosites” (see patents and references cited above), and as described in further detail, below.

The term “cDNA” as used herein includes a nucleic acid or oligonucleotide derived from a naturally occurring message (mRNA) or having a sequence substantially the same as a naturally occurring message (mRNA).

The term “hybridize” or “hybridization” includes, in addition to standard hybridization (e.g., Watson-Crick base pairing), Hoogsteen-based double or triple helix formation, reverse Hoogsteen hydrogen bonding interactions, and the like. See, e.g., Giovannangeli (1997) Antisense Nucleic Acid Drug Dev. 7:413-421; U.S. Patent Nos. 5,473,060; 6,004,750. Hoogsteen triple-helical three-way junctions (ternary complexes) can be designed from the same sequences used for the Watson-Crick triple-helical three-way

junctions, see, e.g., Husler (1994) Arch. Biochem. Biophys. 313:29-38; Husler (1995) Arch. Biochem. Biophys. 322:149-166. For example, the invention can comprise oligonucleotide probes (also called foldback triplex-forming oligonucleotides) that can hybridize to single-stranded complementary polypurine nucleic acid targets by Hoogsteen base pairing as well as by Watson-Crick base pairing (see, e.g., Kandimalla (1995) Nucleic Acids Res. 23:1068-74). Nucleic acid analogs can also be used to design the oligonucleotide probes of the invention, e.g., peptide nucleic acids (PNAs) and analogues of peptide nucleic acids can be designed to form duplex, triplex, and other structures with nucleic acids, see, e.g., U.S. Patent No. 5,986,053.

The term "nucleic acid" as used herein refers to a deoxyribonucleotide or ribonucleotide in either single- or double-stranded form. The term encompasses nucleic acids, i.e., oligonucleotides, containing known analogues of natural nucleotides which have similar or improved binding properties, for the purposes desired, as the reference nucleic acid. The term also encompasses nucleic-acid-like structures with synthetic backbones. DNA backbone analogues provided by the invention include phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, morpholino carbamate, and peptide nucleic acids (PNAs); see Oligonucleotides and Analogues, a Practical Approach, edited by F. Eckstein, IRL Press at Oxford University Press (1991); Antisense Strategies, Annals of the New York Academy of Sciences, Volume 600, Eds. Baserga and Denhardt (NYAS 1992); Milligan (1993) J. Med. Chem. 36:1923-1937; Antisense Research and Applications (1993, CRC Press). PNAs contain non-ionic backbones, such as N-(2-aminoethyl) glycine units, and can be used as probes (see, e.g., U.S. Patent No. 5,871,902). Phosphorothioate linkages are described in WO 97/03211; WO 96/39154; Mata (1997) Toxicol. Appl. Pharmacol. 144:189-197. Other synthetic backbones encompasses by the term include methyl-phosphonate linkages or alternating methylphosphonate and phosphodiester linkages (Strauss-Soukup (1997) Biochemistry 36:8692-8698), and benzylphosphonate linkages (Samstag (1996) Antisense Nucleic Acid Drug Dev 6:153-156). Modified internucleoside linkages which are resistant to nucleases are described by, e.g., U.S. Patent No. 5,817,781. The term nucleic acid is used interchangeably with the terms gene, cDNA, mRNA, probe and amplification product.

The terms "oligonucleotide" or "oligonucleotide probe" as used herein defines a molecule comprised of more than three deoxyribonucleotides or ribonucleotides or equivalents thereof. The oligonucleotides can occur naturally as in a purified restriction digest or be produced synthetically. In the device and methods of the present invention, the oligonucleotide probes can be at least greater than about 10 mer in length, about 12 mer in length, about 20-mer in length, about 25 mer in length, or can range from about 10 to 30 to 50 mer, or greater. The probes of the invention can be linear or circular or stem-loop. For example, single-stranded circular oligonucleotides can bind to both single-stranded and double-stranded target nucleic acids by Watson-Crick base pairing or Hoogstein associations, as discussed below; see, e.g., U.S. Patent Nos. 5,683,874; 5,674,683; 5,514,546.

The term "sample of nucleic acid targets" or "sample of nucleic acid" as used herein refers to a sample comprising DNA or RNA, or nucleic acid representative of DNA or RNA isolated from a natural source, in a form suitable for hybridization or association (e.g., as a soluble aqueous solution) to another nucleic acid or polypeptide or combination thereof (e.g., immobilized probes). The nucleic acid may be isolated, cloned or amplified; it may be, e.g., genomic DNA, mRNA, or cDNA from substantially an entire genome, substantially all or part of a particular chromosome, or selected sequences (e.g. particular promoters, genes, amplification or restriction fragments, cDNA, etc.). The nucleic acid sample may be extracted from particular cells or tissues. The cell or tissue sample from which the nucleic acid sample is prepared is typically taken from a patient suspected of having a genetic defect or a genetically-linked pathology or condition, e.g., a cancer, associated with genomic nucleic acid base substitutions, amplifications, deletions and/or translocations. Methods of isolating cell and tissue samples are well known to those of skill in the art and include, but are not limited to, aspirations, tissue sections, needle biopsies, and the like. Frequently the sample will be a "clinical sample" which is a sample derived from a patient, including sections of tissues such as frozen sections or paraffin sections taken for histological purposes. The sample can also be derived from supernatants (of cells) or the cells themselves from cell cultures, cells from tissue culture and other media in which it may be desirable to detect chromosomal abnormalities or determine amplicon copy number. In some cases, the nucleic acids may be amplified using standard techniques such as PCR, prior to the hybridization or association to the immobilized array probe. In alternative embodiments, the target nucleic

acid may be unlabeled, or labeled (as, e.g., described herein) so that its binding to the probe (e.g., oligonucleotide, or clone, immobilized on an array) can be detected. The probe can be produced from and collectively can be representative of a source of nucleic acids from one or more particular (preselected) portions of, e.g., a collection of polymerase chain reaction (PCR) amplification products; or, the probe can be representative of a chromosome or a chromosome fragment, or a genome.

The term "solid substrate" or "substrate surface" as used herein is a solid or "semi-solid" material which can form a solid support for the array device of the invention. The substrate surface can be selected from a variety of materials including, e.g., polyvinyl, polystyrene, polypropylene, polyester, other plastics, glass, $Si0_2$, other silanes, hydrogels, gold or platinum, and the like; see further examples described, below. The solid surfaces are amino-derivatized, as described below.

The terms "hybridizing specifically to" and "specific hybridization" and "selectively hybridize to," as used herein refer to the binding, duplexing, or hybridizing of a nucleic acid molecule preferentially to a particular nucleotide sequence under stringent conditions. The term "stringent conditions" refers to conditions under which a probe will hybridize preferentially to its target subsequence, and to a lesser extent to, or not at all to, other sequences. A "stringent hybridization" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization (e.g., as in array, Southern or Northern hybridizations) are sequence dependent, and are different under different environmental parameters, as described in further detail below.

Nucleic Acids and Oligonucleotide Probes

This invention provides devices and methods for use in the detection and/or isolation of nucleic acids. The devices and methods of the invention use non-covalently immobilized nucleic acid or oligonucleotide probes. These probes can be made and expressed *in vitro* or *in vivo*, any means of making and expressing nucleic acids and probes used in the devices or practiced with the methods of the invention can be used. The invention can be practiced in conjunction with any method or protocol known in the art, which are well described in the scientific and patent literature.

The nucleic acid sequences of the invention, e.g., probes of the devices, whether, e.g., RNA, cDNA, fragments of genomic DNA, can be isolated from a variety of

sources, genetically engineered, amplified, and/or expressed recombinantly. Any recombinant expression system can be used, including, in addition to mammalian cells, e.g., bacterial, yeast, insect or plant systems.

Alternatively, these nucleic acids can be synthesized *in vitro* by well-known chemical synthesis techniques, as described in, e.g., Carruthers (1982) Cold Spring Harbor Symp. Quant. Biol. 47:411-418; Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896; Narang (1979) Meth. Enzymol. 68:90; Brown (1979) Meth. Enzymol. 68:109; Beaucage (1981) Tetra. Lett. 22:1859; U.S. Patent No. 4,458,066. Double stranded DNA fragments may then be obtained either by synthesizing the complementary strand and annealing the strands together under appropriate conditions, or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Techniques for the manipulation of nucleic acids, such as, e.g., generating mutations in sequences, subcloning, labeling probes, sequencing, hybridization and the like are well described in the scientific and patent literature, see, e.g., Sambrook, ed., MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

Nucleic acids can be analyzed and quantified by any of a number of general means well known to those of skill in the art. These include, e.g., analytical biochemical methods such as NMR, spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and hyperdiffusion chromatography, various immunological methods, e.g. fluid or gel precipitin reactions, immunodiffusion, immuno-electrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immuno-fluorescent assays, Southern analysis, Northern analysis, dot-blot analysis, gel electrophoresis (e.g., SDS-PAGE), RT-PCR, quantitative PCR, other nucleic acid or target or

signal amplification methods, radiolabeling, scintillation counting, and affinity chromatography.

Oligonucleotide primers can be used to generated device probes or to amplify an "associated" or hybridized nucleic acid for detection or replication purposes. These techniques can also be used for site-directed mutagenesis (e.g., to generate alternative probes). Amplification methods are also well known in the art, and include, *e.g.*, polymerase chain reaction, PCR (PCR PROTOCOLS, A GUIDE TO METHODS AND APPLICATIONS, ed. Innis, Academic Press, N.Y. (1990) and PCR STRATEGIES (1995), ed. Innis, Academic Press, Inc., N.Y., ligase chain reaction (LCR) (see, e.g., Wu (1989) Genomics 4:560; Landegren (1988) Science 241:1077; Barringer (1990) Gene 89:117); transcription amplification (see, e.g., Kwoh (1989) Proc. Natl. Acad. Sci. USA 86:1173); and, self-sustained sequence replication (see, e.g., Guatelli (1990) Proc. Natl. Acad. Sci. USA 87:1874); Q Beta replicase amplification (see, e.g., Smith (1997) J. Clin. Microbiol. 35:1477-1491), automated Q-beta replicase amplification assay (see, e.g., Burg (1996) Mol. Cell. Probes 10:257-271) and other RNA polymerase mediated techniques (*e.g.*, NASBA, Cangene, Mississauga, Ontario); see also Berger (1987) Methods Enzymol. 152:307-316; Sambrook; Ausubel; U.S. Patent Nos. 4,683,195 and 4,683,202; Sooknanan (1995) Biotechnology 13:563-564.

In practicing the methods of the invention, samples of nucleic acid are hybridized to immobilized probes nucleic acid on arrays. In one embodiment, the hybridization and/or wash conditions are carried out under moderate to stringent conditions. An extensive guide to the hybridization of nucleic acids is found in, e.g., Sambrook Ausubel, Tijssen. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on an array or a filter in a Southern or northern blot is 42°C using standard hybridization solutions (see, e.g., Sambrook), with the hybridization being carried out overnight.

Stringent hybridization conditions can include, e.g., hybridization in a buffer comprising 50% formamide, 5x SSC, and 1% SDS at 42°C, or hybridization in a buffer comprising 5x SSC and 1% SDS at 65°C, both with a wash of 0.2x SSC and 0.1% SDS at 65°C. Exemplary stringent hybridization conditions can also include a hybridization in a buffer of 40% formamide, 1 M NaCl, and 1% SDS at 37°C, and a wash in 1X SSC at 45°C. Alternatively, hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1X SSC/0.1% SDS at 68°C can be used to identify and isolate nucleic acids within the scope of the invention. Those of ordinary skill will readily recognize that alternative but comparable hybridization and wash conditions can be utilized to provide conditions of similar stringency. However, the selection of a hybridization format is not critical, as is known in the art, it is the stringency of the wash conditions that set forth the conditions which determine whether a nucleic acid is within the scope of the invention. Wash conditions used to identify nucleic acids within the scope of the invention include, e.g.: a salt concentration of about 0.02 molar at pH 7 and a temperature of at least about 50°C or about 55°C to about 60°C; or, a salt concentration of about 0.15 M NaCl at 72°C for about 15 minutes; or, a salt concentration of about 0.2X SSC at a temperature of at least about 50°C or about 55°C to about 60°C for about 15 to about 20 minutes; or, the hybridization complex is washed twice with a solution with a salt concentration of about 2X SSC containing 0.1% SDS at room temperature for 15 minutes and then washed twice by 0.1X SSC containing 0.1% SDS at 68°C for 15 minutes; or, equivalent conditions. Stringent conditions for washing can also be, e.g., 0.2 X SSC/0.1% SDS at 42°C. In instances wherein the nucleic acid molecules are deoxyoligonucleotides ("oligos"), stringent conditions can include washing in 6X SSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos). See Sambrook, Ausubel, or Tijssen (cited below) for detailed descriptions of equivalent hybridization and wash conditions and for reagents and buffers, e.g., SSC buffers and equivalent reagents and conditions.

Solid Substrates

The invention provides associate/ hybridization devices comprising non-covalently immobilized nucleic acid or oligonucleotide probe on a positively charged solid substrate. A positive charged surface is a surface with a net positive charge. It can be a

surface that is cationic, or positively charged only under specific, defined conditions. The positive charge can be completely or partially derived from the material making up the solid substrate or because the solid surface has been derivatized with a composition that has a net positive charge (or can be induced to have a positive charge under certain conditions). The surface can be of a rigid, semi-rigid ("semi-solid") or a flexible material. The surface can be flat or planar, be shaped as wells, raised regions, etched trenches, pores, beads, filaments, or the like. Solid substrates can be of any material upon which a nucleic acid or an oligonucleotide can be immobilized, as described herein. For example, suitable materials can include paper, glass, ceramics, quartz or other crystalline substrates (e.g. gallium arsenide), metals, metalloids, polyacryloylmorpholide, various plastics and plastic copolymers such as Nylon™, Teflon™, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polystyrene/latex, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, various membranes and gels (e.g., silica aerogels), paramagnetic or superparamagnetic microparticles (see, e.g., U.S. Patent No. 5,939,261) and the like.

In one embodiment, a glass surface is used. The glass can comprise a planar or curved glass surface, a glass bead, a porous glass surface or a surface comprising a plurality of glass fibers. See, e.g., U.S. Patent Nos. 6,054,022; 5,843,767; 5,462,642; 5,447,604; 5,409,573.

As noted above, in one embodiment, a surface (e.g., glass) is derivatized with a composition comprising a net positive charge. Reactive functional groups that can react with amino groups to generate a positively charged composition can be, e.g., hydroxyl or carboxyl groups, or the like. Silane (e.g., mono- and dihydroxyalkylsilanes, aminoalkyltrialkoxy-silanes, 3-aminopropyltriethoxysilane, 3-aminopropyltrimethoxysilane) can provide a hydroxyl functional group for binding to a solid substrate surface and a positively charged moiety in the form of an amine functional group. In one specific embodiment, the solid substrate is glass derivatized with an amino-derivatized silane (see Figure 1B).

Oligonucleotide or cDNA probes are non-covalently adsorbed onto specific areas, or "biosites" on the array surface. After electrostatic association of the probes to the array, the remaining positive charges not associated with a probe nucleic acid, i.e., a

"residual" positive charge, is "capped" by reaction with a second composition, e.g., by acylation, or electrostatically neutralized with a composition comprising a negative charge, or, "masked" with an adsorbed composition having no net positive or negative charge (a "neutral" composition), e.g., a neutral lipid detergent or a neutral high MW polymer.

Positively charged solid surfaces

In one embodiment, the methods of the invention comprise depositing solutions of nucleic acids onto amino-derivatized solid surfaces. The amino- moiety can be any amino-chemical group, such as an aminosilane group or equivalent. The solid surfaces include any surface covalently or non-covalently conjugated with an amino (e.g., aminosilane) moiety or equivalent For example, U.S. Patent No. 6,057,040 describes the making of an alkine-bridged bis-(aminosilane) or tris-(aminosilane)-derivatized solid surface. U.S. Patent No. 6,020,028 describes the aminosilane equivalents 3-aminopropyltriethoxysilane and N-(2-aminoethyl)-3-aminopropyltrimethoxysilane. U.S. Patent No. 5,760,130 describes aminating the glass substrate with an aminosilane. See also, U.S. Patent No. 5,350,800.

Other positively charged array surfaces that can be used in the methods of the invention include cationic polyacrylamides, including, e.g., acrylamide/ aminoethylmethacrylate, methylaminoethylmethacrylate, trimethylaminoethylmethacrylate aminohexylmethacrylate, aminopropyl acrylamide, methyl aminopropyl acrylamide, dimethyl aminopropyl acrylamide or trimethyl aminopropyl acrylamide, or mixtures thereof or equivalents thereof. See, e.g., U.S. Patent No. 4,104,226. The cationic polyacrylamide can also comprise N,N,N-trimethylammoniumpropylmethacrylamide, vinyl pyridine/methacrylamidopropyltrimethylammonium chloride, dimethyldiallylammonium chloride, diethyldiallylammonium chloride, 2-methacryloxy-2-ethyltrimethylammonium chloride, trimethylmethacryloxyethylammonium methsulfate, 2-acrylamido-2-methylpropyltrimethylammonium chloride, vinylbenzyltrimethylammonium chloride; see, e.g., U.S. Patent No. 4,584,339.

Cationic hydrogels can be used in the methods of the invention, e.g., a hydrogel with a poly(vinyl alcohol) with poly (ally-biguanido-co-allylamine) hydrochloride. Cationic hydrogels based on hydroxyalkyl acrylates and methacrylates are described, e.g., in U.S. Patent No. 4,163,092; 4,060,678.

Other surfaces include, e.g., diaminocyclohexane (DACH) plasma polymer surface. See, e.g., Lassen (1997) J. Colloid Interface Sci. 186:9-16, describing three different plasma polymer surfaces prepared from hexamethyldisiloxane (PP-HMDSO), acrylic acid (PP-AA), and 1,2-diaminocyclohexane (PP-DACH). See also Malmsten (1997) J. Colloid Interface Sci. 193:88-95.

Another useful surface is a polystyrene treated by oxidative amination. Also useful are high MW polyester resins, described in, e.g., U.S. Patent No. 6,063,895; and, high MW acrylized epoxy resins, acrylisized polyesters, polyesters containing vinyl ether or epoxy groups, and also polyurethanes and polyethers, described in, e.g., U.S. Patent No. 6,048,660.

Surfaces adsorbed or chemically bound with any positively charged proteins can be used. For example, protamines, polyamines (e.g., spermine), homopolyamino polypeptides (e.g., polyornithine, polylysine, polyarginine) or co-polyamino polypeptides (e.g., co-poly-(Lys, Ala) etc.) acids.

Surfaces derivatized with cationic lipids, e.g., lipids or detergents with trimethylammonium groups, can also be used. Detergent polyamines are described in, e.g., U.S. Patent No. 6,008,316.

Nucleic acids and/or oligonucleotides are deposited onto the positively charged surface to form an "array," a plurality of biosites, as described above. As defined above, a "biosite" as used herein means a discrete area, spot or site on the surface of an array with immobilized probes attached thereto. In the methods of the invention, the nucleic acid or oligonucleotide probes are non-covalently attached to a positively charged array surface. Arrays made by and used in the methods of the invention, as is typical of "biochips" or arrays, comprise a plurality of such spots or sites (see patents and references cited above). Each biosite on the arrays made by and used in the methods of the invention, also as is typical of arrays, can have a single specie of probe attached thereto, e.g., a plurality of nucleic acids (e.g., cDNA) or oligonucleotides having substantially the same sequence. Under appropriate conditions, an association or hybridization can occur between the immobilized probe and a target (e.g., a "sample") molecule. The maximum number of biosites per array, or reaction chamber, will depend on the size of the array or reaction vessel and on the practical optical resolution of an accompanying detector/imager. For example, an

array of 16 (4 × 4 array) biosites may be deposited on a substrate or base material that eventually forms the bottom of the entire reaction vessel. Each biosite would comprises a circle of approximately 25 to 200 microns (μm) in diameter. Thus, for a 16 biosite array, each of the 16 × 200 μm diameter area contains a uniform field of probes immobilized to the array substrate (base material) in a concentration which is highly dependent on the probe size and the well size; as well as the method used to immobilize the probe, as described below). In this exemplary array, each 25 to 200 μm diameter area can contain millions of probe molecules. Also, each of the 16 different biosites (probe sites) can contain one type of probe. Thus, 16 different probe types can be assayed in an array containing 16 biosites (4 × 4 array) per reaction chamber. As another example, four separate 10×10 arrays (400 biosites) can be generated to fit into one well of a 96 well microtiter plate with sufficient spacing between each of the 400 biosites. For this 10×10 format, 400 hybridization experiments are possible within a single reaction chamber corresponding to 38,400 (96 × 400) assays/hybridization that can be performed nearly simultaneously.

The nucleic acids or oligonucleotides can be "spotted" onto the positively charged surface by any means, see, e.g., Balch, et al., U.S. Patent No. 6,083,763.

"Capping," Neutralizing or Masking Positive Charges

In the methods of the invention, oligonucleotide or cDNA probes are non-covalently adsorbed onto "biosites" on the positively charged array surface. After electrostatic association of the probes to the array, the remaining positive charges not associated with a probe nucleic acid are "capped" by reaction with a second composition or are electrostatically neutralized with a composition comprising a negative charge or are "masked" with an adsorbed composition having no net positive or negative charge (a "neutral" composition).

For a positively charged surface covered with a monolayer of adsorbed probe, if there were a high density of positive charges (e.g., amines) on the surface, not involved in direct interaction with probe, electrostatic attraction between target and the surface could become a dominating force, thereby overwhelming the contribution of duplex formation. Poor base sequence selectivity would be seen in that instance. Thus, the invention provides ways to "cap" the excess of unused surface cations, while retaining tight adsorptive interaction between probe and the surface. The invention provides are four general ways to

achieve this (see also, Summary, above):

a) chemically; by reaction of residual positive charges (e.g., amines) on the array surface with different capping reagents. Standard chemistries can be used, such as acylation reactions, or, reactions to produce Schiff bases. Depending on the type of acylating or other reagents used (e.g., aldehydes or ketones or equivalents), the final reacted product (after reaction with the residual positive charges, e.g., amines) can be, e.g., a carboxamide, a sulfonamide, a urea, a thiourea, a Schiff base, or the like, or a mixture thereof;

b) electrostatically neutralizing positive charge; by using negatively charged compositions, including polymers with a net negative charge. Examples include, e.g., non-complimentary DNA (cDNA), such as salmon sperm DNA; polysulfates, negatively charged proteins, such as dry milk or casein, or combinations thereof or equivalents thereof;

c) increasing distance (reducing electrostatic attraction) between positive surface and hybridization mixture; by "masking" positive charge with neutral polymers, which are adsorbed to the array surface. Examples include detergent solutions (e.g., Tween-20), serum albumin (e.g., BSA or HSA), or a traditional blocking polymer mixture (e.g., 1 mg/ml each of Ficoll, polyvinyl pyrrolidone and bovine serum albumin, "Denhardt's solution"); and

d) using high pH buffers; buffers with a pKa of about 9 to 9.5, which transform positive charge of ammonia $NH_3(+)$ into neutral amine NH_2 by proton abstraction.

In one exemplary embodiment, the residual positively charged surface was "masked" by a combination of a traditional blocking polymer mixture with high pH buffer. The blocking, or "masking," mixture was: 1mg/ml each of Ficoll, polyvinyl pyrrolidone and bovine serum albumin, 5x "Denhardt's solution." This created a non-covalent coating to effect a reduction of electrostatic interaction between unused surface amines and solution state target. The high pH buffer, by increasing solution state pH, reduced the array surface charge as the pK of surface bound amine groups was approached. It was demonstrated that a pre-hybridization step at 150 mM sodium carbonate buffer at pH 9.5 and 5x Denhardt's solution, followed by hybridization in the same buffer, produces a surface capable of high selectivity and high affinity nucleic acid hybridization.

Arrays, or "BioChips"

The invention provides improved methods to make and use "arrays" or "microarrays" or "DNA arrays" or "nucleic acid arrays" or "biochips" and improved variations of making and using known arrays, e.g., GeneChips®, Affymetrix, Santa Clara, CA. As defined above, arrays are generically a plurality of "biosites," each comprising a defined amount of one or more nucleic acid molecules, or probes. The probes are immobilized a solid surface for hybridization to sample nucleic acids. The immobilized nucleic acids can contain sequences from specific messages (e.g., as cDNA libraries) or sequences representative of specific genes, chromosomes or genomes. Other biosites can contain reference sequences and the like. The biosites of the arrays may be arranged on the solid surface at different sizes and different densities. The target element densities will depend upon a number of factors, such as the nature of the label, the solid support, and the like. Each target element may comprise substantially the same nucleic acid sequences, or, a mixture of nucleic acids of different lengths and/or sequences. Thus, for example, a target element may contain more than one copy of a cloned piece of DNA, and each copy may be broken into fragments of different lengths. The length and complexity of the nucleic acid fixed onto the target element is not critical to the invention. See, e.g., Balch, et al., U.S. Patent No. 6,083,763.

EXAMPLES

The following example is offered to illustrate, but not to limit the claimed invention.

Example 1: Array-based Nucleic Acid Hybridization Using Covalently Associated Oligonucleotide Probes

The following example demonstrates that the methods of the invention provide an improved and efficient means to generate nucleic acid arrays.

To demonstrate the efficiency of probes non-covalently associated to arrays using the methods of the invention, the adsorptive strategy of the invention was compared to standard covalent strategies for immobilizing nucleic acid probes to arrays.

For covalent attachment, amino-modified oligonucleotides were placed upon an epoxy-silanized surface by deposition of 25 nL of oligonucleotide at 200 μM in 15 mM NaOH using a Hamilton Micro Lab 2200™ robot. A high concentration of the applied

oligonucleotide is required in this covalent coupling reaction to compensate for the slow reaction of primary amines with an epoxide moiety in water. The adsorptive attachment chemistry was performed by deposition of a solution in distilled water at concentrations in the 25 μM to 0.2 μM range.

For adsorptive attachment, slides were silanized with 3-aminopropyl-trimethoxysilane in vapor phase in a vacuum oven: 70° C at 25 in. Hg, overnight. Oligonucleotides were deposited as 25nL solutions in distilled water at 5 μM. After drying, "unused" amino-surface was capped with acetic anhydride in vapor phase (50°C, 22 in. Hg, 1 hour (h)) followed by capping with 0.5 M succinic anhydride in DMF (25C, 1 h).

The saturability and stability of oligonucleotide adsorption to the amino-modified surface was determined. 25 nL spots of a radio-labeled 12-mer target complimentary to the wild type K-ras 12-mer probe, at concentrations from 0.01 to 40 μM were deposited. Microarrays were formed, one each per well, in 8x12 well microtiter format. The excess of deposited oligonucleotide was then washed off with repeated water washing and bound surface density determined with a storage phosphorimager.

For titration of the aminosilanized surface with a 12-mer oligonucleotide, the DNA 12-mer, corresponding to wild type K-ras 1, 5'-CGCCACCAGGTC-3' (SEQ ID NO:1), was ^{33}P labeled with polynucleotide kinase using standard methods, see, e.g., Sambrook. Chemical capping or adsorptive capping with 5x Denhardt's solution was employed prior to hybridization. Perfectly matched pairing used the K-ras1 target to k1 probe. A single base GT mismatch used the K-ras 1 target to the k3 probe. $Y=n*k[T]/(1+k[T])$ was used for fitting of the binding curves, where $Y=T/P$ is the bound target probe ratio, T is the number of target molecules bound/mm^2 and P is the number of probe molecules/mm^2; [T] is free target concentration in solution; k is the affinity constant; and n the value of Y at saturation.

For chemically capped microarrays, oligonucleotide targets were hybridized and washed at pH 7.5 or 9.5. For uncapped microarrays, 5x Denhardt's solution was applied to the array in water (10min.) followed by a prehybridization solution containing 150 mM sodium bicarbonate, 5x Denhardt's solution, pH 9.5 (10 minutes). Hybridization solution (^{33}P labeled CGCCACCAGGTC (SEQ ID NO:1), 150mM sodium bicarbonate, 5x Denhardt's solution, pH 9.5) was applied for 2 hours, 25C. The array was washed two times

(10 min at 25C) in 150 mM sodium bicarbonate, 5x Denhardt's solution, pH 9.5. Hybridization was detected with a Cyclone™ phosphorimager. Microarray capture probes: codon *12*: k1: 5'-gacctggtggcg-3' (SEQ ID NO:2); k2: 5'-gacctagtggcg-3' (SEQ ID NO:3); k3: 5'-gacttggtggcg-3' (SEQ ID NO:4); k4: 5'-gacctcgtggcg-3' (SEQ ID NO:5); k5: 5'-gacctgatggcg-3' (SEQ ID NO:6); k6: 5'-gacctgctggcg-3' (SEQ ID NO:7); k7: 5'-gacctgttggcg-3' (SEQ ID NO:8).

For single nucleotide polymorphism analysis with electrostatically attached probes and covalently attached probes: 152 base pair (bp) K-ras amplicons were obtained by PCR. Wild type amplicon (K-ras 1, SEQ ID NO:1) was obtained by amplification of a commercial genomic DNA source (Sigma, St. Louis, MO). K-ras 2 (SEQ ID NO:3) and K-ras 7 (SEQ ID NO:8) mutants were obtained by amplification of human genomic DNA from cell lines A549 and SW480, respectively. PCR primers were 5' -labeled with digoxigenin and had the sequence: 5'-dig-actgaatataaacttgtggtagttggacct-3' (SEQ ID NO:9) and 5'-dig-tcaaagaatggtcctgcacc -3' (SEQ ID NO:10). Chaperone oligonucleotide, 5' - taggcaagagtgccttgacgatac-3'-dig (SEQ ID NO:11) forms duplex with target immediately proximal to the target-probe hybridization site and holds the target into a locally opened state, thereby minimizing undesired side effects of target secondary structure. *Hybridization conditions*: Prehybridization solution, containing 150 mM sodium citrate, 5X Denhardt's solution, pH 8.0, was applied to the array for 10 minutes. It was vacuumed off and hybridization solution (1 nM amplicon, 0.1 μM chaperone, 150 mM sodium citrate, 5X Denhardt's solution, pH 8.0) was applied to the array. After 2 hours of hybridization, the array was washed two times in 100 mM sodium citrate pH7.5, 10 minutes each. Amplicon was detected using anti-digoxigenin antibody linked to alkaline phosphatase (Boehringer Mannheim) and development by enzyme linked fluorescence (Molecular Probes), as described by Haugland, R.P. *Handbook of Fluorescent Probes and Research Chemicals* (Molecular Probes, Inc., ed. 6th, 1996, pp.117-120. Fluorescence intensities were detected with an Alfa Imager 2000™, processed using GeneView 1.0™ (Genometrix Genomics, Inc., The Woodlands, TX), Microsoft Excel 97™, and Sigma Plot 3.0™ software.

Such analysis revealed a saturating probe density of $3x10^{11}$ molecules/mm^2. Assuming that one 12-mer oligonucleotide occupies a surface area of about 600 sq. angstroms (that is, 10 angstrom width & 5 angstrom rise per base repeat), it was calculated

that the maximum number of oligonucleotide molecules needed to form a closely packed monolayer on the surface would be 2×10^{11} molecules/mm^2. Thus, comparison of experimental and calculated density gives evidence that a densely packed monolayer of oligonucleotides is forming during the adsorption process. A similar adsorption analysis with a radiolabeled 36-mer probe was complete. Maximum saturable probe density with the 36-mer was found to be 0.6×10^{11} molecules/mm^2. Comparison to the density calculated for a closest packed 36 mer monolayer (0.6×10^{11} molecules/mm^2) suggests that a densely packed probe film can be formed for probes as long as 36 bases.

It was next determined how many oligonucleotide molecules can be bound adsorptively to the amino-modified surface. 10 nL of radio-labeled 36mer at 8 different concentrations from 23 to 0.18 µM was deposited to all wells of a standard amino-modified 96 well glass slide. Excess (non-deposited) oligonucleotides were washed off with water. Bound surface density was determined by imaging with a Cyclone Phosphoroimager.™

It was found that saturation of the surface was achieved at approximately 9 µM of oligonucleotide concentration under conditions where the spot size was 0.85 mm^2. Knowing the total volume, concentration and spot area of the printed oligonucleotide, it was calculated that the maximal adsorbed probe density was approximately 0.11 pmole/mm^2, corresponding to 6.6×10^{10} molecules/mm^2. Assuming that one 35mer oligonucleotide occupies a surface area of 10 x 180 angstroms, it was calculated that the maximum number of oligonucleotide molecules needed to form a monolayer on the surface would be 5.6×10^{10} molecules/mm^2. Comparison of experimental and theoretical numbers indicates that a densely packed monolayer of oligonucleotides is formed during the adsorption process.

It was further determined that in the presence of salt in the "printing" (probe depositing) solution does not interfere with probe (oligonucleotide) adsorption. A higher pH (0.1 M $NaHCO_3$ at pH 7.9 also does not interfere with oligonucleotide adsorption because aliphatic amines on the surface are still protonated below pH 8. This described adsorptive method is also insensitive to oligonucleotide concentration variation so as long as the solution state concentration of oligonucleotide exceeds that required to form the limiting monolayer, which is typically in the 10 to 20 µM range.

If the surface was dried after printing, subsequent treatment with high salt concentrations (about 3 M NaCl or 1 M sodium phosphate) or high pH (up to about pH 11.5)

near room temperature did not displace the attached probe monolayer. The loss of radioactivity was less than 10 % in both instances. Noticeable removal (more than 50 %) of radio-labeled probe from the adsorbed monolayer was found only during washing at elevated temperatures (95°C) with high salt (1 M sodium phosphate).

For a positively charged surface covered with a monolayer of adsorbed probe, if there were a high density of amines on the surface, not involved in direct interaction with probe, electrostatic attraction between target and the surface could become a dominating force, thereby overwhelming the contribution of duplex formation. Poor base sequence selectivity would be seen in that instance. Thus, the invention sets forth novel means to "cap" the excess of unused surface cation, while retaining tight adsorptive interaction between probe and the surface.

To make a slightly negatively charged surface, arrays were covalently capped with acetic anhydride in the vapor phase, then capped them again with 0.5 M succinic anhydride in DMF at room temperature for 1 h.

The utility of a traditional blocking polymer mix was also tested. The mix was: 1mg/ml each of Ficoll, polyvinyl pyrrolidone and bovine serum albumin, "5xDenhardt's solution" as a non-covalent coating to effect a reduction of electrostatic interaction between unused surface amines and solution state target. Additional fine-tuning of target interaction was obtained by an increase of solution state pH, so as to reduce surface charge as the pK of surface bound amine groups is approached. Thus, the invention provides a novel method to prepare an array for hybridization: a pre-hybridization step at 150 mM sodium carbonate buffer at pH 9.5 and 5xDenhardt's solution, followed by hybridization in the same buffer, produces a surface capable of high selectivity and high affinity nucleic acid hybridization.

These two methods of capping were compared by performing analytical hybridization of a radiolabeled synthetic 12mer K-ras gene target to a microarray containing its Watson crick complement and a related probe which differed by a single base change. The covalent method of capping produced a binding isotherm with an apparent dissociation coefficient near to 1×10^{-8} M, saturating near to 0.5 targets bound per probe equivalent. These data are not significantly affected by pH in the range from 7.0 to 9.5. For comparison, at pH 9.5 and in the presence of Denhardt's solution as the blocking agent, the measured dissociation constant is also seen to be approximately 1×10^{-8} M, suggesting that as expected,

the covalent capping process had not altered the nucleic acid probe in any way that could be detected *via* hybridization.

At the highest K-ras target concentration, the limiting capacity to bind a synthetic 12mer target appears to saturate at a slightly higher value for non-covalent blocking with Denhardt's (0.8 targets bound per probe equivalent, as compared to about 0.5), but considering the standard error of the data, this apparent difference is not significant.

That secondary consideration aside, the data suggest that the two methods of excess surface charge neutralization are seen to be nearly equivalent in terms of target binding affinity and binding capacity per unit area. For both methods of capping, the capacity to form duplex with a short synthetic target is characterized by single nucleotide mismatch discrimination.

The above adsorptive probe attachment chemistry (with covalent capping) was used to perform single base polymorphism analysis on a 152 base pair K-ras PCR product. These results were compared with arrays prepared using covalent attachment of amine-modified probes to an epoxysilane coated surface. Results obtained using amplicon targets derived from the K-ras gene show that hybridization achieved using adsorptive probe binding and chemical capping is comparable in affinity and specificity to the best that could be obtained with covalently attached probes on a neutral epoxysilane surface. As seen in both instances, the relatively stable G-T mismatch (K-ras 1 target amplicon with k3 probe) generates signals that are diminished by a factor of ten relative to perfectly matched 12- base pair pairing (K-ras 1 target with k1 probe). Discrimination for all other single base mismatches is measured to be significantly greater than a factor of twenty five.

A number of embodiments of the invention have been described. Neverthe-less, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of producing an array comprising a plurality of discrete biosites, wherein the biosites comprise non-covalently attached nucleic acids, comprising the following steps:

(a) providing a solid surface comprising a net positive charge or derivatized with a composition comprising a net positive charge;

(b) providing at least one solution comprising a nucleic acid comprising a net negative charge;

(c) depositing a solution of step (b) onto a discrete biosite on the solid support of step (a), wherein the nucleic acid becomes non-covalently attached to the solid support of step (a) at least in part by an electrostatic attraction between the negatively charged nucleic acid and the positively charged solid support; and,

(d) contacting the solid support of step (c) with a composition that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid.

2. The method of claim 1, further comprising drying the solid support after step (c) and before step (d).

3. The method of claim 1, wherein the solid surface comprises a glass surface.

4. The method of claim 3, wherein the glass surface comprises a planar glass surface, a glass bead, a porous glass surface or a surface comprising a plurality of glass fibers or equivalents thereof.

5. The method of claim 1, wherein the solid surface comprises a nitrocellulose or a nylon membrane or equivalents thereof.

6. The method of claim 1, wherein substantially all of the solid surface of step (a) is positively charged.

7. The method of claim 1, wherein the solid surface of step (a) is positively charged substantially only on a plurality of discrete biosites.

8. The method of claim 1, wherein the solid surface of step (a) comprises an amino-derivatized solid surface.

9. The method of claim 8, wherein the amino-derivatized solid surface comprises an aminosilane-derivatized solid surface.

10. The method of claim 9, wherein the aminosilane-derivatized solid surface comprises an alkine-bridged bis-(aminosilane) or tris-(aminosilane) or an equivalent thereof or a mixture thereof.

11. The method of claim 9, wherein the aminosilane comprises a 3-aminopropyltriethoxysilane, a N-(2-aminoethyl)-3-aminopropyltrimethoxysilane or an equivalent thereof or a mixture thereof.

12. The method of claim 1, wherein the solid surface of step (a) comprises a cationic polyacrylamide surface.

13. The method of claim 12, wherein the cationic polyacrylamide surface comprises an acrylamide/aminoethylmethacrylate composition, a methyl-aminoethylmethacrylate, a trimethylaminoethylmethacrylate, an amino-hexylmethacrylate, an aminopropyl acrylamide, a methyl aminopropyl acrylamide, a dimethyl aminopropyl acrylamide or a trimethyl aminopropyl acrylamide, or mixtures or equivalents thereof.

14. The method of claim 1, wherein the solid surface of step (a) comprises a cationic hydrogel.

15. The method of claim 14, wherein the cationic hydrogel comprises a poly(vinyl alcohol) - poly (ally-biguanido-co-allylamine) hydrochloride composition.

16. The method of claim 1, wherein the solid surface of step (a) comprises a diaminocyclohexane (DACH) plasma polymer.

17. The method of claim 1, wherein the solid surface of step (a) comprises a polystyrene treated by oxidative amination.

18. The method of claim 1, wherein the solid surface of step (a) comprises a solid surface derivatized with a positively charged protein

19. The method of claim 18, wherein the positively charged protein comprises a protamine, a polyamine, a homopolyamino polypeptide or a co-polyamino polypeptide or an equivalent thereof.

20. The method of claim 19, wherein the protein comprises a spermine, a polyornithine, a polylysine, a polyarginine, or a co-poly-(Lys, Ala) acid or an equivalent thereof.

21. The method of claim 1, wherein the solid surface of step (a) comprises a solid surface derivatized with a cationic lipid or detergent or an equivalent thereof.

22. The method of claim 21, wherein the cationic lipid or detergent comprises a trimethylammonium group.

23. The method of claim 1, wherein the solution of step (b) comprises an aqueous solution.

24. The method of claim 23, wherein the aqueous solution consists essentially of distilled water.

25. The method of claim 1, wherein the nucleic acid comprises a cDNA or a genomic DNA.

26. The method of claim 25, wherein the cDNA or genomic DNA comprises a length of between about 100 bases to about 800 bases.

27. The method of claim 1, wherein the nucleic acid comprises an oligonucleotide.

28. The method of claim 27, wherein the oligonucleotide comprises a length of between about 8 bases to about 80 bases.

29. The method of claim 28, wherein the oligonucleotide comprises a length of between about 10 bases to about 15 bases.

30. The method of claim 1, wherein the concentration of nucleic acid in the aqueous solution is between about 0.2 μM to about 25 μM.

31. The method of claim 30, wherein the concentration of nucleic acid in the aqueous solution is between about 5 μM to about 20 μM.

32. The method of claim 31, wherein the concentration of oligonucleotide in the aqueous solution is between about 9 μM to about 10 μM.

33. The method of claim 1, wherein the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid neutralizes the positive charges by a covalent reaction.

34. The method of claim 33, wherein the composition forms a Schiff base with a positively charged composition on the surface of the array.

35. The method of claim 34, wherein the composition comprises an aldehyde or a ketone.

36. The method of claim 33, wherein the positive charge-neutralizing composition comprises an acylating reagent.

37. The method of claim 36, wherein reaction of the acylating reagent with a positively charged composition on the solid support produces a carboxamide, a sulfonamide, a urea or a thiourea.

38. The method of claim 36, wherein the acylating reagent comprises an acetic anhydride or a butyric anhydride.

39. The method of claim 38, wherein the acetic anhydride or the butyric anhydride is in a vapor phase.

40. The method of claim 39, wherein the reaction conditions of step (d) comprise a temperature of about 50°C and an atmospheric pressure of about 22 mM of Hg.

41. The method of claim 40, wherein the reaction conditions last for about 8 to 18 hours.

42. The method of claim 1, further comprising the step of reacting the solid support of step (d) with a succinic anhydride or equivalent thereof.

43. The method of claim 42, wherein the reaction conditions comprise 0.5 M succinic anhydride in DMF at room temperature for about one hour.

44. The method of claim 36, wherein the acylating reagent comprises a methylisothiocyanate or equivalent thereof.

45. The method of claim 1, wherein the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid of step (d) neutralizes by electrostatic neutralization.

46. The method of claim 45, wherein the composition comprises a high molecular weight polymer comprising a net negative charge.

47. The method of claim 46, wherein the high molecular weight polymer comprises a polysaccharide or equivalent thereof.

48. The method of claim 46, wherein the high molecular weight polymer comprises a polysulfate or equivalent thereof.

49. The method of claim 46, wherein the high molecular weight polymer comprises dry milk or casein or equivalent thereof.

50. The method of claim 46, wherein the high molecular weight polymer comprises a nucleic acid with a net negative charge.

51. The method of claim 50, wherein the nucleic acid with a net negative charge is salmon sperm DNA.

52. The method of claim 45, wherein the composition comprises a basic pH buffer.

53. The method of claim 52, wherein the buffer has a pH of between about 9 and 9.5.

54. The method of claim 45, wherein the composition comprises a detergent comprising a net negative charge.

55. The method of claim 1, wherein the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid neutralizes the positive charge by non-covalent and non-electrostatic adsorption to the solid surface, thereby masking substantially all of the exposed positive charges on the array surface.

56. The method of claim 55, wherein the composition comprises a high molecular weight polymer with no net positive or negative charge.

57. The method of claim 55, wherein the composition is Denhardt's solution or an equivalent thereof.

58. The method of claim 55, wherein the composition comprises a neutral detergent solution or an equivalent thereof.

59. The method of claim 55, wherein the composition comprises a serum albumin or an equivalent thereof.

60. The method of claim 55, wherein the composition comprises a polyvinylpyrrolidone or an equivalent thereof.

61. The method of claim 60, wherein the polyvinylpyrrolidone comprises a N-vinyl pyrrolidone, a 3-methyl N-vinylpyrrolidone, a N-vinyl amide pyrrolidone, a N-vinyl acetate pyrrolidone, a vinylpyrrolidone -vinyl acetate copolymer, or an acrylamide-vinylpyrrolidone co-polymer, or an equivalent thereof or a mixture thereof.

62. A nucleic acid array comprising a solid surface comprising a plurality of discrete biosites comprising a non-covalently associated nucleic acid produced by a method comprising the following steps:

(a) providing a solid surface comprising a net positive charge or derivatized with a composition comprising a net positive charge;

(b) providing at least one solution comprising a nucleic acid comprising a net negative charge;

(c) depositing a solution of step (b) onto a discrete biosite on the solid support of step (a), wherein the nucleic acid becomes non-covalently attached to the solid support of step (a) at least in part by an electrostatic attraction between the negatively charged nucleic acid and the positively charged solid support; and,

(d) contacting the solid support of step (c) with a composition that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid.

63. The DNA array of claim 62, wherein the array surface onto which the nucleic acid has been deposited is coated with a monolayer of adsorbed, non-covalently attached nucleic acid.

64. The DNA array of claim 63, wherein the monolayer of adsorbed, non-covalently attached oligonucleotides has an oligonucleotide density of about 10^7 to about 10^{12} molecules per mm^2.

65. The DNA array of claim 64, wherein oligonucleotide density is about 10^{11} molecules per mm^2.

66. The DNA array of claim 62, wherein the nucleic acid comprises a cDNA or a genomic DNA.

67. The DNA array of claim 66, wherein the cDNA or genomic DNA comprises a length of between about 100 bases to about 800 bases.

68. The DNA array of claim 62, wherein the nucleic acid comprises an oligonucleotide.

69. The DNA array of claim 68, wherein the oligonucleotide comprises a length of between about 8 bases to about 80 bases.

70. The DNA array of claim 68, wherein the oligonucleotide comprises a length of between about 10 bases to about 15 bases.

71. The DNA array of claim 62, wherein the solid surface comprises a glass surface.

72. The DNA array of claim 71, wherein the glass surface comprises a planar glass surface, a porous glass surface or a surface comprising a plurality of glass fibers or equivalent thereof.

73. The DNA array of claim 62, wherein the solid surface comprises a nitrocellulose or a nylon membrane or equivalent thereof.

74. The DNA array of claim 62, wherein the composition of step (d) that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid neutralizes the positive charges by a covalent reaction.

75. The DNA array of claim 62, wherein the positive charge-neutralizing composition comprises an acylating reagent or equivalent thereof.

76. The DNA array of claim 63, wherein the acylating reagent comprises an aldehyde or a ketone or equivalent thereof.

77. The DNA array of claim 63, wherein the acylating reagent comprises an acetic anhydride or a butyric anhydride or equivalent thereof.

78. The DNA array of claim 63, wherein reaction of the acylating reagent with a positively charged composition on the solid support produces a carboxamide, a sulfonamide, a urea or a thiourea or equivalent thereof.

79. A method for determining if a nucleic acid in a test sample can hybridize to a nucleic acid immobilized onto an array comprising the following steps:

(a) providing a test sample comprising a nucleic acid;

(b) providing a nucleic acid array comprising a solid surface comprising a plurality of discrete biosites comprising a non-covalently associated nucleic acid produced by the following steps:

(i) providing a solid surface comprising a net positive charge or derivatized with a composition comprising a net positive charge;

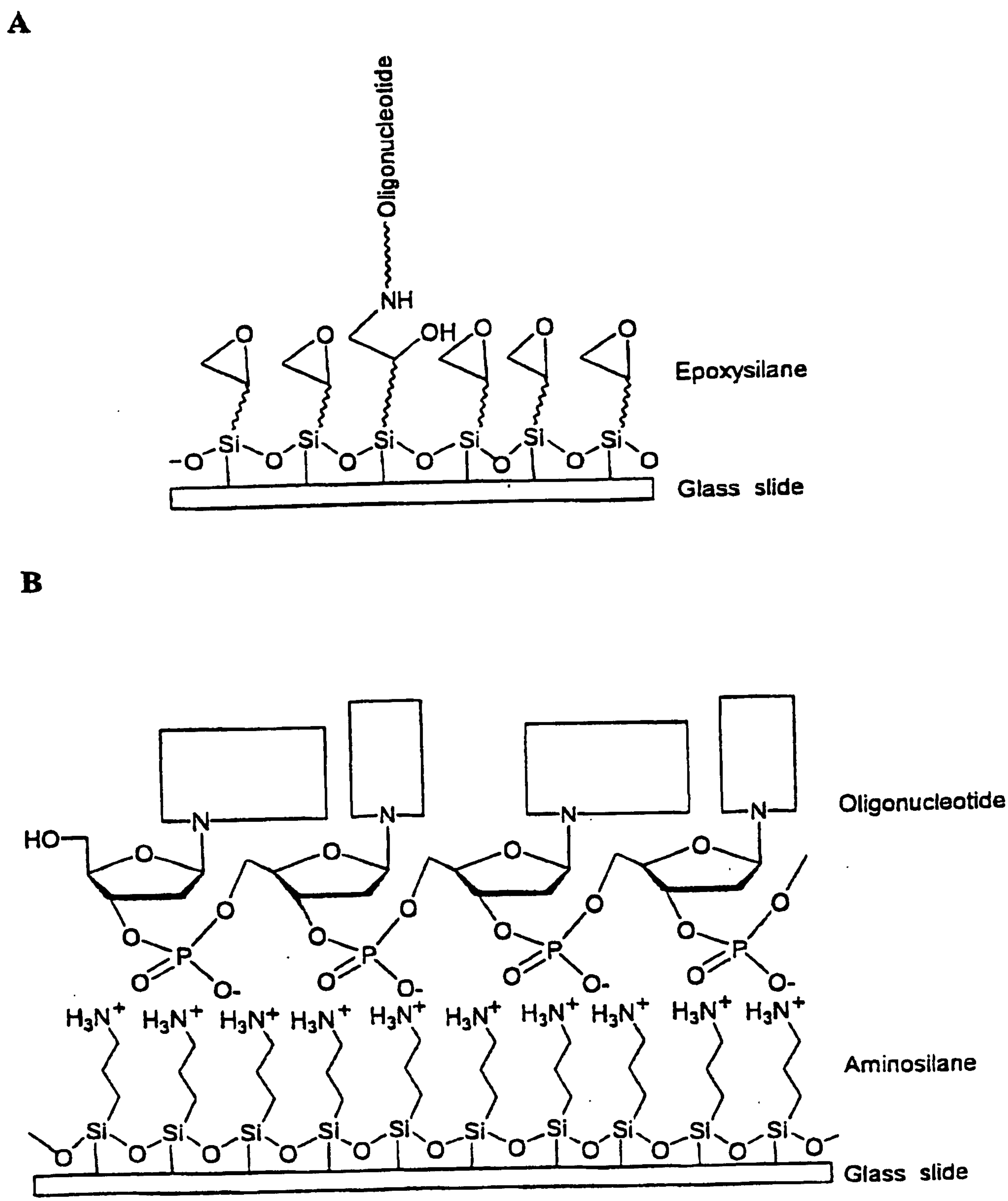
(ii) providing at least one solution comprising a nucleic acid comprising a net negative charge;

(iii) depositing a solution of step (ii) onto a discrete biosite on the solid support of step (a), wherein the nucleic acid becomes non-covalently attached to the solid support of step (a) at least in part by an electrostatic attraction between the negatively charged nucleic acid and the positively charged solid support, and,

(iv) contacting the solid support of step (iii) with a composition that neutralizes substantially most of the positive charges on the solid support not associated with the non-covalently attached nucleic acid; and

(c) contacting the test sample of step (a) with the DNA array of step (b); and

(d) determining if a nucleic acid in the test sample hybridizes to a nucleic acid immobilized onto an array.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OH
Epoxysilane
Si
O
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P
O-
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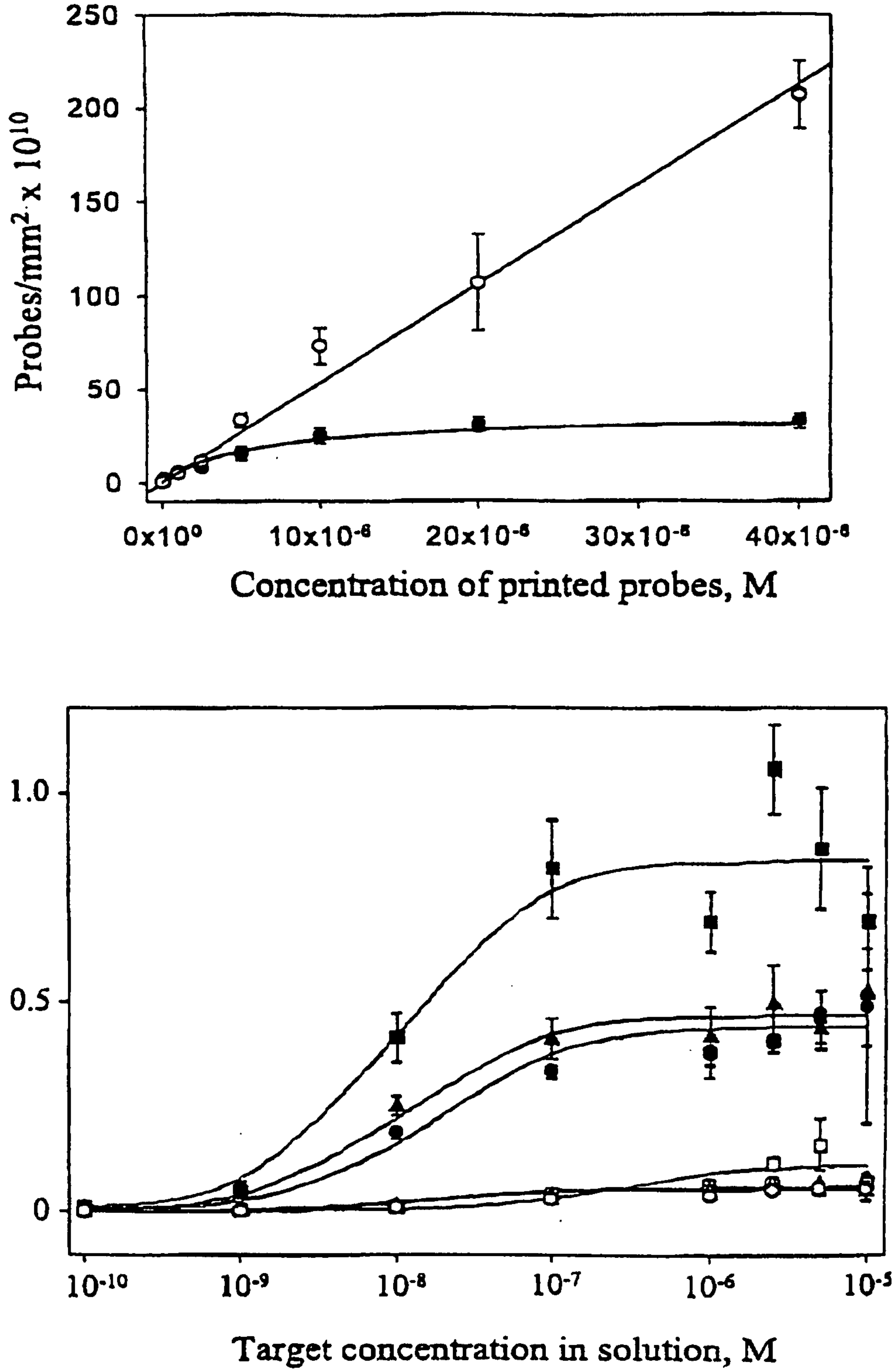
Aminosilane
Si
Glass slide

Fig 1

A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50
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40x10^{-6}
Concentration of printed probes, M
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0.5
0
T/P
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10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
Target concentration in solution, M

Fig. 2